Sample Identification, Chemical Composition Analysis and Testing of Physical State of the Sample Using Spectra Obtained At Different Sample Temperatures

The present invention relates to a method of identifying components of a sample at different temperatures. More specifically, this invention provides a method of sample identification, chemical composition analysis, and testing the physical state of a sample by analyzing spectra obtained from a sample subjected to different temperatures.

BACKGROUND OF THE INVENTION

Material objects produce or absorb electromagnetic radiation in certain parts of the spectrum of electromagnetic radiation. Spectral distribution of the produced radiation by an object, or effect of the object on the spectrum of the radiation following interaction with the object, depends on the chemical composition of the object, its physical state (for example, but not limited to gas, liquid, solid), and existing physical conditions acting on the object (for example but not limited to, pressure, temperature). Under certain conditions spectral characteristics of the radiation produced by an object, or changes in the spectrum of radiation interacting with the object can be used for identifying chemical elements and compounds in the object, or for identifying physical conditions acting on the object. While spectral specificity of the radiation produced by an object, or spectral specificity of change introduced to radiation interacting with an object, may be used for identification or the analysis of the chemical composition of an object, the specificity of spectral changes under different conditions, temperature in particular, is not recognized and, as a result, is not used in practice.

Currently, emission, absorbance and Raman spectroscopy are used to extract information from a sample using spectral measurements of electromagnetic radiation of the sample. Each of these methods, under certain condition can be used for identification of chemical elements and compounds of a sample, or for the determination of a relative or absolute, concentration of the elements and compounds in the sample.

It is well known that a chemical element generates or absorbs electromagnetic radiation in a characteristic set of narrow spectral bands distributed in a specific fashion within the electromagnetic radiation spectrum, including its visible part, when the element is in a form of free atoms and heated to sufficiently high temperature. The pattern of these bands can be used for identifying chemical elements present in the sample. After suitable calibration, information on relative intensity of radiation or absorption levels at these bands can be used for measuring the relative concentration of one or more elements in the sample under investigation. Furthermore, after additional calibration, when volume of sample is normalized, the absolute concentration of the element in the sample may be determined.

Substances containing molecular compounds also produce specific images of emission and absorption bands due to transition between different vibration modes of molecules. Since the amount of energy needed to change a vibration mode of molecule is much lower than that needed to change electron energy level within the atom, emission and absorption bands of molecules are usually placed in middle and far infrared bands of the spectrum. Detection of such bands is difficult and emission spectra of molecules are seldom recorded. However, various indirect detection methods for measurement of absorption spectra may be used, for example Raman spectroscopy, which allows detection of transitions between different vibration modes of molecules.

Transition between vibration modes of molecule which are normally responsible for long wavelength absorption and emission bands can be excited by radiation with shorter wavelengths, thereby producing overtone absorption bands in regions of the near infrared, or visible regions of the spectrum, which are easier to detect. Unfortunately, the spectral patterns associated with these bands are not as easy to interpret as those obtained from free atoms, and their use for identification of molecules requires further data processing using methods such as PLS (partial least squares), PCA (principle components analysis) or neural networks.

Even with application of these advanced data processing methods, identification and recognition of the molecular composition of a complex substance is

not an easy task, and there still exists a need for methods that produce well defined spectrum-dependent patterns, that are specific for a particular chemical compound, and that can be used for identification of chemical compounds

Another limitation of presently used methods of absorption spectroscopy relates to the manner in which measurements are performed. Spectrum radiation affected by a light-absorbing sample may be determined by two factors: the spectral characteristics of radiation used for measurement, and the absorbing properties of the tested sample. To ensure that measurement results are independent of the spectral characteristics of the used radiation, the result needs to be suitably corrected. Such a correction is possible when spectral characteristics of used radiation are known. Since properties of all radiation sources can change with time, sometimes quite rapidly, the spectral characteristics of used radiation needs to be measured at a time that is close to the time of sample measurement. Furthermore, it is preferred that this measurement is made using the same measurement equipment as that used for the measurement of radiation affected by the sample. Once these two measurements are taken, the impact of samples on the radiation can be identified as a ratio of spectral distribution of radiation affected by the sample to that of radiation incident on the sample. The negative decimal logarithm of this ratio is called the spectral optical density of the sample and it can be used for identification of one or more chemical compounds. The spectral optical density may also be used, with further processing, to determine the relative or absolute concentration of one or more compounds in a sample. This can be determined with acceptable precision if the sample average absorbance is relatively small and if the differences in the absorbance in bands where radiation is absorbed, and non-absorbed, are large enough to provide a sufficient signal to noise ratio. For highly absorbing samples, this condition usually cannot be satisfied, and calculation of spectral optical density cannot be performed with required precision. To resolve this problem additional attenuators, that are not affected by radiation, are used for sample illumination, but the use of attenuators influences the precision of the measurement. Therefore, there exists a need for a method of measurement that eliminates the requirement for characterizing radiation that is used for testing.

It is known that observation of the mesomorphic phases of a substance is a difficult task because of a lack of sufficiently sensitive methods for identifying different phases. It is also known that absorption spectra of a chemical compound depends not only on the molecular composition of the compound but also on the physical state of the sample (gas, liquid, solid), and the mesomorphic phase of the substance. Differences in the spectra of substances in different mesomorphic phases are very small and they are typically attributed to temperature changes required for phase transition of the sample. As a result spectroscopy has not been used for analysis of phase state of samples. Therefore there exists a need for a measurement method for easy and relatively simple identification of phase transition in the molecular substances.

It is an object of the invention to overcome disadvantages of the prior art.

The above object is met by the combinations of features of the main claims, the sub-claims disclose further advantageous embodiments of the invention.

SUMMARY OF THE INVENTION

The present invention relates to a method of identifying components of a sample at different temperatures. More specifically, this invention provides a method of sample identification, chemical composition analysis, and testing the physical state of a sample by analyzing spectra obtained from a sample subjected to different temperatures.

The present invention provides a method for identifying a chemical compound, analyzing a chemical composition or investigating a physical change of a sample, the method comprising obtaining spectra of electromagnetic radiation of the sample at two or more than two different temperatures.

The present invention also provides a method (A) for identifying a chemical compound, analyzing a chemical composition or investigating a physical change of a sample comprising:

i)   collecting one or more than one spectrum of radiation of the sample at a first temperature and averaging the result to obtain a first spectrum, and obtaining one or more than one spectrum of radiation of the sample at one or more than one second temperature, and averaging the result at each temperature, to obtain one or more than one second spectrum;

iii)   calculating a difference in absorbance between the first and the one or more than one second spectrum; and

iv)   using the difference in the absorbance, identifying a chemical compound, analyzing a chemical composition or investigating a physical changes of the sample.

The invention also pertains to the method as just defined (Method A), wherein in the step of calculating (step iii)), a negative decimal logarithm of a ratio of radiation intensity registered for the sample at the one or more than one second temperature to a radiation intensity obtained at the first temperature, is determined.

This invention embraces method (Method A) as defined above wherein in the step of calculating (step iii)) comprises

a)   determining a mean value of registered spectra for each wavelength measured for the fist spectrum and the one or more than one second spectrum;

a)   calculating the standard deviation for each wavelength; and

b)   obtaining a ratio of the mean value to the standard deviation.

The present invention also provides the method as defoined above (Method A), wherein in the step of collecting (step i)), the temperature of the sample continuously changes between the first temperature, and the one or more than one second temperature. Alternatively, in the step of collecting (step i)), the temperature of the sample can be changed in discrete manner between the first temperature and the one or more than one second temperature. 9. Furthermore, in the step of collecting (step i)), the first temperature and the one or more than one second temperature of the sample can be adjusted outside of, or inside of, a sample compartment. 10. The first temperature and the one or more than one second temperature of the sample may result from radiation that is used for spectral analysis. The first temperature can be lower than the one or more than one second temperature, or the first temperature can be higher than the one or more than one second temperature.

The present invention also pertains to the method as defined above (Method A), wherein, in the step of calculating (step iii)), a running average, a standard deviation, and a relative thermal spectral change are calculated for sets of measurements, where each set of measurements is collected at the one or more than one second temperature, and a thermal dynamic of change in the sample is determed.

This invention embraces the method defined above (Method A) wherein, in the step of collecting (step i)), a spectral analyzer is used to obtained the first spectrum and the one or more spectrum, the spectral analyzer selected from the group consisting of a grating scanning spectrometer, a prism scanning spectrometer, a grating and prism spectrometers with detector arrays, a Fourier transform spectrometer, a filter switching spectrometer, a tuneable filter spectrometer, a Fabry-Perot scanning spectrometer, an acousto-optical tuneable filter and an instrument allowing for spectrum analysis.

According to the present invention there is provided a method for chemical composition analysis of a sample using spectra obtained from a sample at different temperatures. In one embodiment, which is provided here by way of an example only, the method consists in multiple repetition of the following steps:
- bringing the sample temperature to a certain level,
- placing the sample in the spectrometer,
- illuminating the sample with electromagnetic radiation in required spectral range,
- collecting of one or more spectra of radiation subjected to interaction with the sample at set temperature and repeating the above steps at least one more time at different sample temperature.

As is evident to those of skill in the art, the methods defined above can be performed in many different ways, provided that a set of spectra of the electromagnetic radiation obtained following interaction with the sample obtained at two or more than two different temperatures is collected. In the case of samples of complex molecular composition, it has been observed that the spectral absorbance of the sample depends on temperature, therefore spectra obtained at different temperature have different spectral content. Once spectra of the sample at different

temperatures are obtained the resulting data can be processed in different ways as required.

This invention overcomes existing limitations in the art of spectral measurement methodology and provides a method for conducting spectral measurements and data processing resulting in a specific response. The methods described herein do not require measurements of spectral intensity distribution of radiation used for illumination of the sample during absorption measurements.

Furthermore, the present invention overcomes existing limitations of in the recognition of phase transition of a chemical compound, and provides a spectral measurement methodology based on analysis of thermal dynamics of absorption spectra of a compound using the dependence of the thermal dynamic of the absorption spectra on temperature range in which the absorbance spectra are collected.

As described in more detail below, changes in spectra of substances caused by change of temperature have been observed to be substance specific, and these spectra can be also used for identifying chemical compounds in a sample, or for composition analysis of chemically complex samples.

This summary does not necessarily describe all necessary features of the invention but that the invention may also reside in a sub-combination of the described features.

BRIEF DESCRIPTION OF THE DRAWINGS

Features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

Figure 1 shows a block diagram of a typical spectroscopic system.

Figure 2 shows a flow chart of standard measurement process leading to the production of an absorption spectrum of a sample, and that can be used for chemical sample identification and analysis.

Figure 3 shows spectra of different samples obtained using a spectrometer and prior art methods. Figure 3A shows the spectrum of water. Figure 3B shows the spectrum of 50% methanol solution in water. Figure 3C shows spectrum of methanol.

Figure 4 shows a flow chart of measurement processes leading to the production of an absorption difference created by changes in sample temperature.

Figure 5 shows changes of absorbance of a sample obtained while gradually warming up a sample from refrigerator (about 4°C) to room temperature (about 20°C). Figure 5A shows the spectrum of water. Figure 5B shows the spectrum of 50% methanol solution in water. Figure 5C shows spectrum of methanol.

Figure 6 shows a scatter plot of measured (predicted) concentrations against reference data for a mixture of intralipid in water in the presence of two interfering analytes calculated from a difference of absorption measured at two different temperatures. Three independent sets of data with correlation coefficient between all three components below 0.2 were used for model development, model verification and method validation.

Figure 7 shows a scatter plot of measured concentrations against reference data for protein concentration in animal sera calculated from a difference of absorption measured at two different temperatures.

Figure 8 shows a scatter plot of measured concentrations against reference data for cholesterol concentration in animal sera calculated from a difference of absorption measured at two different temperatures.

Figure 9 shows a scatter plot of measured concentrations against reference data for bilirubin concentration in animal sera calculated from a difference of absorption measured at two different temperatures.

Figure 10 shows a scatter plot of measured concentrations against reference data for sodium ions concentration in animal sera calculated from a difference of absorption measured at two different temperatures.

Figure 11 shows a flow chart of a measurement process leading to the production of a spectral response representing an average thermal change and the thermal dynamic of a spectral signal. The obtained value reaches a maximum in spectral bands where absorbance demonstrates the highest stability (i.e. the smallest variability).

Figure 12 shows examples of the spectral stability of absorbance spectra of samples measured as described herein. The spectra were calculated as a ratio of the averaged spectral intensity of radiation affected (in this particular case transmitted) by the sample for a defined period of time, during which temperature of the sample was gradually increasing, to the standard deviation of the variability of the spectral intensity of the radiation during the same time period. Figure 12A shows the thermal spectral stability of water. Figure 12B shows the thermal spectral stability of 50% methanol solution in water. Figure 12C shows the thermal spectral stability of methanol.

Figure 13 shows spectral stability of a water sample over about two minute time period as measured using the method described herein, with about a half minute time between the end of one measurement and beginning next measurement. Figure 13A shows the relative thermal spectral stability for water at the beginning of the experiment. Figure 13B shows the relative thermal spectral stability for water at about 2 min. Figure 13C shows the relative thermal spectral stability for water at about 4 min. Figure 13D shows the relative thermal spectral stability for water at about 6 min. Figure 13E shows the relative thermal spectral stability for water at about 8 min.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of sample identification and chemical composition analysis of a sample at different temperatures. More specifically, this invention provides a method of sample identification, chemical composition analysis, and testing the physical state of a sample by analyzing spectra obtained from a sample subjected to different temperatures. This invention also pertains to the field of spectral measurements of an object containing a complex molecular composition. More specifically, this invention relates, but is not limited, to methods of identifying chemical compounds, analyzing chemical compositions, and observing physical changes in a sample absorbing electromagnetic radiation. In particular, the present invention provides methods for identifying a chemical compound, analyzing the chemical composition of a sample, and testing the physical state of a sample using spectra obtained at different temperatures of the sample.

The following description is of a preferred embodiment by way of example only and without limitation to the combination of features necessary for carrying the invention into effect.

With reference to Figure 1, there is shown a typical spectrometric instrument which is not to be considered limiting in any manner, comprising:
- a stable source of electromagnetic radiation (101), for example but not limited to a lamp;
- a radiation beam forming component (102), for example, but not limited to, mirrors or lenses, for efficient collection of radiation from the source (101) and for forming a radiation beam that can be used for sample illumination. Filters (not shown) may be used for removal of unwanted radiation as required;
- a shutter (103) to block or open a path of the radiation beam to a sample (1042);
- a sample compartment (104) equipped with a sample holder (1041) to hold the sample (1042) in a desired position. The sample compartment, or sample holder may be equipped with temperature measuring and controlling elements (1043), for temperature monitoring and to keep a constant sample temperature during

measurement, or to change the temperature in a controlled fashion by heating or

cooling;

- radiation collecting optics (105), for example consisting of lenses or mirrors to

collect radiation affected by the sample, to form a radiation beam of a required shape,

and to direct the beam for further analysis;

- a spectrum analyzer (106) that decomposes the delivered radiation beam received

from the collecting optics (105) into particular spectral components and directs them

to a radiation detector (107). While any instrument capable to decompose delivered

radiation into spectral components, and measure the intensity of these spectral

components can be used as the spectrum analyzer, a non-limiting example of a

spectrum analyzer is a grating spectrometer that angularly disperses radiation of

different wavelengths in different directions, so that particular spectral components

are registered with separate detectors, for example, in a form of a detector array.

Non- limiting examples of a spectral analyzer include grating scanning

spectrometer, a prism scanning spectrometer, a grating and prism spectrometers with

detector arrays, a Fourier transform spectrometer, a filter switching spectrometer, a

tuneable filter spectrometer, a Fabry-Perot scanning spectrometer, an acousto-optical

tuneable filter, and any instrument that allows for spectrum analysis of a sample.

The signal from the radiation detector (107) can be preprocessed or processed

with a suitable electronic circuit (108), converted into a digital form by means of A/D

converter (109), and directed to computer (110), which can process, store, display or

send obtained information to an external user (111) for further utilization. The same

computer (110) can be also used to perform numerous other functions, for example

verification of calibration and performance of the analyzer, and control the function of

the analyzer for example, but not limited to, opening and closing shutter (103),

verification of sample (1042) presence in the sample compartment (104), or

measurement and control of sample temperature (1043) and the like.

Spectral absorbance measurement with such the instrument consists of several

steps, as shown, but not limited to, in Figure 2. Instrument preparation for

measurement (step 2-1) typically involves verifying, in a trial run, the functionality of

the instrument and measurement parameters, for example amplification or integration

time, slit width, scanning speed, measurement time, sample temperature or other criteria as required. Once working parameters of instrument are determined and set, one or more than one reference spectral measurements of the radiation beam is obtained, without a sample present in the optical path, and stored in computer memory (2-2). However, an attenuator, that replaces the sample, may be present for this step (2-2). Immediately before or after reference measurement RL (2-2), one or more than one measurement of instrument internal noise RD may be obtained and stored (2-3) using the same setting of all system parameters (as for step 2-2), with exception that the shutter is in the closed position to block radiation from the source to detector. The sample is introduced into the optical path (step 2-4), and the settings of the instrument are modified to accommodate instrument performance as required by attenuated by the sample radiation level, and one or more than one light SL and dark SD measurements are performed (steps 2-5 and 2-6). The data is stored for further processing and analysis.

Usually light measurements (2-5) are conducted either at room temperature or after the sample reaches a temperature set by a sample temperature controlling system. Variation in sample temperature during measurement is undesirable and is typically considered as a factor that affects the precision of measurement. After four measurements (steps 2-2, 2-3, 2-5 and 2-6; RL, RD, SL and SD, respectively) are collected results of the same kind of measurements (if more than one measurement was obtained) are averaged (step 2-7; 2-7a - reference light, 2-7b - reference dark, 2-7c – sample light and 2-7d - sample dark) and dark signals are subtracted from the light signal (step 2-8; 2-8a - for reference measurement and step 2-8b – for sample). Both measurements, the reference measurement and sample measurement (2-8a and 2-8b) are normalized for amplification, or integration times (if different amplifications or integration times were applied during these measurements), and the absorbance is calculated (step 2-10) as a decimal logarithm of ratio of spectral intensity of radiation affected, and not affected, by the sample. Obtained spectra can be used either for identification, or concentration, of a chemical compound (step2-11) with results presented, as in step 2-12, or the results used for measurement of the concentration of the compound of interest in the sample (steps 2-21 to 2-27). This includes collection of thermal absorbance difference for larger number of samples (2-21), development of statistical model (2-22), collection a large number of sample data for model validation

(2-23), model validation (2-24), collection of data for samples to be analyzed (2-25), prediction of concentration of compound of interest using developed models (2-26) and result presentation (2-27)

Examples of absorption spectra obtained, using the method outlined in Figure 2, of three different substances: water, a 50% solution of methanol in water, and pure methanol are shown in Figures 3 A, B and C respectively. While obtained spectra show some differences it is clear that they contain only limited number of specific features that can be used to make the spectra of these substances easily distinguishable, especially in comparison to another similar substance.

When properly configured, and the samples to be analyzed obey Beer's law, the absorbance data can be used for sample identification or, after suitable calibration, for analysis of sample composition (steps 2-11 and 2-12). In many cases, especially for light scattering samples such a simple procedure may not be sufficient, and more advanced measurement methods are required. These methods include: collecting data from larger set of samples (2-21) and development of one or more statistical models (steps 2-22 to2-24) that represent the relation between measured spectral values and the concentrations of analytes of interest in the sample; applying one of many available mathematical methods for example, but not limited to, Partial Least Square fitting (PLS), or Principal Components Analysis (PCA) or Neural Network and using the developed method; the model validation, using statistically justified set of independent measurements (2-24) and predicting in step 2-26 the concentration of the analytes for which the mathematical models have already been built, using data obtained in step 2-25. Results can be presented (2-27).

As will be recognized by one of skill in the art, the order of spectral data collection steps 2-2 to 2-6 may be varied, and that the required data can be collected in any other order. For example, which is not to be considered limiting, a sample measurement can be taken before reference and dark measurements. Also the number of measurements obtained, the order of data averaging, or the normalization of the data can be performed in any order without departure from the spirit of the method.

According to an aspect of the present invention, there is provided a method for chemical composition analysis and a method for the analysis of physical state of a sample using spectra obtained from a sample at different temperatures.

With reference to Figure 4 there is shown a flow chart of a method performed in accordance with present invention. However it is to be understood that variations in this method may also be employed.

In step 4-1, the instrument is prepared for measurements. This step may include spectrum measurement of a radiation beam in the absence of the sample (as in step 2-2), but this measurement is not required for use in further steps. Also step 2-3 and 2-6 (reference and sample dark measurement, respectively) are not required for use in this method. Step 4-1 may be further modified to set the instrument parameters to values that are optimal for light sample collection. After the instrument is ready, the sample at the preset temperature is introduced into spectrometer (4-2), where the temperature control system either maintains the initial temperature, or it brings the sample temperature to a desired level. At this point one or more than one spectrum (SL1) of radiation affected by the sample is collected (4-3), after which the sample temperature is modified (4-4) using the temperature control system of the spectrometer (1043, Figure 1) or an external temperature controller (not shown in Figure 1), and another one or more than one spectrum (SL2) of radiation affected by the sample at the new temperature is collected (4-6). The spectra in each group are averaged and difference in absorbance of the sample being at these two temperatures is calculated as a negative decimal logarithm or ratio of the measured spectral radiation intensity (4-10). Results obtained in this manner can be used in a similar fashion (steps 4-11 and 4-12 or 4-21 to 4-27) as the sample absorbance measured in the process represented in Figure 2.

When active temperature control of a sample is not available or carried out, alternated methods to conduct measurements can be used. After the instrument becomes ready to obtain measurements (as a result of step 4-1), the sample at a certain temperature, lower or higher than temperature of the instrument, is placed in the measurement compartment of the spectrometer (4-2). For example, which is not to be considered limiting in any manner, the sample may be cooled to refrigerator

temperature, for example from about 0°C to about 8°C, and be introduced into system in step 4-2 for continuous spectrum and temperature collection (step 4-3) until the sample reaches a desired temperature (4-4), for example about 18°C to about 25°C (room or any other temperature). The obtained spectra (4-3; in this case steps 4-4 and 4-5 are omitted, since continuous data is collected during the change in sample temperature), are then divided in subgroups containing, for example, but not limited to, either equal number of measurements, or measurements corresponding to the same temperature change (in this case an increase) during which time the selected group of measurements were collected. All measurements belonging to a single group are then averaged (step 4-7) and a negative decimal logarithm (base 10) of the ratio of averaged values for two selected groups, obtained in a similar fashion, are calculated (4-10). The results obtained using this method represent differences in the averaged absorbance, over selected groups of absorbance of the sample, that arise from the difference of averaged temperature when the corresponding groups of spectra were collected. These measurements may be processed as required (steps 4-11 to 4-12, or steps 4-21 to 4-26) as outlined above with respect to Figure 2. Sets of absorbance differences obtained using this method, for the first and each consecutive group, for the same substances as presented in Figure 3, are shown in Figure 5.

A comparison of spectral absorbance differences (as shown in Figures 5A-C) obtained for large number of different samples for the same temperature gradient demonstrates that the difference in absorbance is sample specific as is evident, for example, with reference to Figures 5A to C, for water, 50% solution of methanol in water and pure methanol, respectively. Theoretical analysis also indicates that these differences follow Beer's law. Therefore, this analysis can be used for chemical composition analysis in a manner similar to the use of absorbance spectra.

An advantage of the approach described herein consists of eliminating a reference measurement, which in some cases, especially in spectroscopic systems that are specific for measuring light scattering samples, is problematic.

The measurements obtained using the present method may be conducted using cooled samples, for example which is not to be considered limiting in any manner, to

refrigerator temperature, and then placing the sample in the compartment of a spectrometer held at room temperature to permit warming of the sample over time. Ten groups of spectral measurements comprising radiation transmitted by a sample housed within a radiation scattering container can be collected for each sample, and the absorbance difference calculated for selected measurement groups, for example the second and ninth groups. The difference between these two groups may then be used for further analysis.

The absorbance difference between the any group of measurements may be used for analysis of the chemical composition of a sample. The difference in absorbance can be used for analysis of chemical composition of sample in similar fashion as the real absorbance of a sample measured as outlined in Figure 2.

To demonstrate the effectiveness of the method as described herein, temperature related differences in absorption have been collected for two sets of samples. A preliminary experiment was conducted using a random combination of intralipid and two interferents solved in water. A second experiment was conducted on samples comprising a combination of nine different animal sera containing physiological analytes in different proportions. In both cases a special care was taken to eliminate possibility of correlation between any two analytes. In both cases a large number of samples were prepared (over 500 samples in the first experiment and over 400 samples in the second), as required for chemometry.

Samples were cooled and placed into the sample holder at room temperature. Spectra of radiation affected by the samples during the period of sample warming, from refrigerator to room temperature, were continuously collected and differences in absorption for the second and ninth groups of spectra calculated as described above. The results were divided in three sets:

      - the first set was used for statistical model development of the relation between absorbance difference and concentration of the analytes of the interest;

      - the second set was used for model validation; and

      - the last set was used for verification of the method described herein.

The results of this analysis are presented in set of scatter plots show in Figures 6 to 10. The abscise on each graph represents the independently measured value of concentration of a compound of interest, while the ordinate shows the value predicted by a developed model from the spectral information obtained in the measurement process as described above.

Figure 6 shows a scatter plot of intralipid concentrations in water in presence of two non-correlated interferents as predicted by a model, against the concentration of intralipid used for sample preparation. This result indicates a very good predictability of intralipid concentration (all measurements line up along the line with slope 1.(45° line), and an acceptable measurement precision, as almost all measurements are contained within +/- 20% error range.

A further analysis with animal sera was undertaken and results for selected analytes are presented in Figures 7 to 10. Figure 7 shows a scatter plot for protein concentration in animal sera, while Figure 8, 9 and 10 present results for cholesterol, bilirubin and sodium ions. While not all analytes can be measured with the same precision, since not all analytes in natural concentrations provide a signal that is sufficient for spectroscopic measurement, the results demonstrate a very good level of predictability – in all cases results line up along line with slope 1.

These results demonstrate that the method described herein, comprising the measurement of spectral differences in sample absorbance caused by temperature differences in a sample, provides information suitable for the measurement of the concentration of chemical components in the sample. This method is also suitable for determine the measurement of the concentration of chemical components within a complex matrix, such as animal sera, without the need for spectral characterization of radiation used for measurement. As in other methods measurement, errors depend on concentration of the component under consideration and the strength of the signal provided, as it is can be seen with reference to Figures 6-10.

The data sets shown in Figures 6 to 10 demonstrate that measurement of the concentration of a chemical compound within a sample within simple or complex

matrices, such as animal sera, may be obtained using differences in absorbance of the samples obtained at different temperatures.

Different information may be derived when the spectral distribution of radiation intensity obtained during a continuous change in the temperature of the sample is averaged and divided by the standard deviation of these variations at each spectral measurement point. If the instrument noise is measured in similar way in the absence of the sample is low, this ratio, called dynamic range of instrument, shows a high value. For non-absorbing samples this ratio would be preserved. For the samples uniformly absorbing radiation across the whole spectrum, the ratio can be scaled down if the same instrument settings are used for spectral intensity distribution, or for determining the radiation affected by the sample, as those used for unaffected radiation. However, as described herein, it was noted that in the presence of the sample, whose temperature varied during measurement, the response is different from that as expected. Furthermore, in portions of spectra where sample absorbance is small, and where no changes in dynamic range were expected, a large variability in dynamic range was observed.

Further study demonstrates that the temperature-affected dynamic range is sample specific and strongly depends on a mean value and the temperature variability range during time when the to be averaged spectra of affected by the sample radiation, are collected. This method can provide information on the position of isosbestic points in a sample spectrum.

Analysis of water demonstrates that its spectrum depends on temperature and that there also exist points were the absorbance spectrum remains virtually constant, which by extension are referred to as thermal isosbestic points. These points were observed as intersections of two or more water absorption spectra taken at different temperatures. To verify the uniqueness of the method described herein, tests were performed using different chemical compounds of organic and non-organic origin. It has been observed that each sample produces a different, specific pattern that is dependent on mean temperature, and the temperature variability range as shown in Figures 12 A-C, and Figures 13 A-E.

As opposed to isosbestic points, the method proposed in the present invention allows for a quantitative characterization of the thermal behavior of absorbing properties of a sample. The proposed ratio shows high values in portions of the spectra where absorbance of the sample is not affected by the sample and the absorbance is stable, and low values where the impact of temperature is large, and where stability of the sample absorbance is low. Therefore, these measurements represent the thermal dynamic of the signal across the spectrum that results from changes in the temperatures of the sample. The thermal dynamic of the signal should reach a maximum at thermal isosbestic points of the sample.

With reference to Figure 11, there is shown a flow chart of measurements that are performed in accordance with present invention to collect data for determination of a thermal dynamic signal of the radiation that is affected by the sample. In the first step the instrument is prepared for measurement (11-1). This process may include measurements needed to evaluate instrument performance, but these measurements are usually not used in the process of characterization the thermal dynamic signal. Step 11-1 can be used to set the instrument parameters to values that are optimal for collection of radiation affected by the sample. After working conditions of the instrument are determined and set, the spectra of radiation affected by the sample are collected as the sample temperature is changed in a controlled manner. As outlined with reference to the analogous step in Figure 4, this step can be performed in many ways depending on requirements, and on available equipment. For example, which is not to be considered limiting, the sample is cooled to refrigerator temperature, from about 0°C to about 8°C, and it is introduced into the sample holder (step 11-2) and the continuous spectrum is collected (11-3) until the sample reaches a desired temperature, for example but not limited to room temperature. The process of changing the temperature may take different amounts of time, depending of thermal properties of sample holder, or if a sample temperature controller used. In the latter case, the dynamic of the process can be adjusted to increase the detail of thermal dynamic to be resolved. Generally speaking, the slower the process, the more details can be revealed. However, it is required that the measuring instrument is stable enough during the time period for the collection and averaging of each group of spectra. Due to the high sensitivity to the temperature and temperature variations, it is

preferred that such spectra have to be collected under controlled thermal conditions. One of skill in the art appreciates that numerous ways of changing the temperature can be realized during or between time periods when consecutive groups of spectra are collected.

The obtained spectra (11-5) are divided in subgroups containing, for example, but not limited to, groups comprising an equal number of measurements, or groups corresponding to the same temperature increase during the time when the selected group of measurement were collected. All measurements belonging to a single group are averaged (11-7) to obtain a mean value for each wavelength. Simultaneously, the standard deviation of variability within the group at each wavelength is calculated (11-8), and for each wavelength, the ratio of the mean to the standard deviation, is calculated (11-20). Alternatively, instead of continuous temperature change, stepwise change can be applied (steps 11-2 to 11-10 in the right column in chart of Figure 11) and the ratio of the mean to the standard deviation can be calculated This data can be presented in a form of a set of graphs (one for each group of measurements), that represent the thermal dynamic of the changes (spectral dependence of these changes will be referred here as the thermodynamic signal) in a spectrum of radiation affected by the sample at a certain mean temperature, and whose temperature changed during measurement within the range determined by measurement system. Comparison of the results obtained for different groups, and registered at different temperatures of samples shows the variability of the thermodynamic signal as function of sample composition, mean temperature and temperature range, over which the spectra were averaged. Because of high sample specificity, the result can be used for identification of chemical compounds (11-21) or used for analysis of spectral thermodynamic (1-31). The result of the analysis may be presented (11-22 and 11-32) or transferred for further use.

Exemplary results of chemical compound, solutions or mixtures, were obtained for water, 50% solution of methanol in water and pure methanol, as shown in Figures 12A, B and C.

These results demonstrate that by using the method described above, spectral thermodynamic signals that depend on mean temperature of the sample during

measurement, and that depend on the range of temperature change during collection of data, may be obtained that are specific for given substance. The graphs (Figures 12A-C) further demonstrate that the thermal spectra contain more pronounced features that vary significantly between samples than absorption spectra shown in Figures 3A-C. The graphs shown in Figure 12 A-C exhibit richer structure than the absorption spectra of Figures 3A-C, including very strong features in spectral ranges where absorption of the sample is relatively weak (e.g. the 600-900 nm range). Therefore results obtained using the method outlined above, and with reference to Figures 4 and 11 are more suitable for identification of chemical compounds than methods associated with obtaining a standard absorption spectrum.

As graphs in Figure 12A-C show, the average spectral thermal dynamic of the signal affected by the sample radiation (or thermodynamic signal) strongly depends on temperature and the temperature variability range used for averaging. These results may therefore be used to identify temperature points where properties of the substances change dramatically for some reason. Without wishing to be bound by theory, the observed changes at temperature points may occur, for example, as a result of a mesomorphic phase transition, or other physical change in the sample, for example a transition from monomer to dimmer and the reverse, or thermal related transitions between different internal structures of solids and liquids. These temperature points where thermodynamic signal undergoes dramatic changes may be a good indicator of changes in internal structure of the sample, for example water, or bonds between water molecules and walls of the vial containing the water ('Drost-Hansen' temperature, for example). Therefore the spectrum analyzing method described herein may be a powerful tool for testing physical changes in a substance.

As one of skill in the art understands, the temperature dependent data can be collected in many different ways for example, but not limited to, by measurement of light affected by the sample at two or more than two distinctive different temperatures, for example one at a low temperature, and the other, or remaining measurements, at any elevated temperature, for example but not limited to from about 0°K to a temperature accessible with present day technology without damage to sample or used measuring apparatus. Selection of a temperature range to be used will

depend upon the sample and the physical state of the sample being analyzed. In this regard, spectra may be obtained spanning any desired temperature range. Measurements can be also taken in reversed order with a first measurement obtained at a higher temperature, and a second and all other consecutive measurements obtained at lower temperatures. Also the transition from one temperature to another, or for averaging measurements from low to high or from high to low, can be performed either continuously or in discrete steps with constant or variable temperature change from one temperature setting to another.

The method of the present invention may be used on any sample for which a chemical composition analysis or analysis of physical state may be desired. For example, but not wishing to be limiting the sample may comprise a body part of a subject, for example, but not limited to a finger, toe, earlobe, arm or back, samples of various natural or synthetic products, self contained or in a container, samples or portions of various products and materials. Alternatively the sample may comprise a solid, semi-solid, fluid, vapor or gas obtained from any source, for example, but not limited to blood, urine, mucus, sweat, lymph, excrement, secretions and the like, dairy products, any form of food or drinks, a chemical product for example but not limited to plastics, products of semiconductor industry, products of petrochemical industry, wood and paper industry products, neutral and hazardous products in loose or contained form, clear and contaminated water, clear or contaminated air, gases or vapors and so on. Samples that are not part of living creature are considered *in vitro* samples, while parts of life forms such as humans, animal, plants, bacteria and all other living creatures, which have to be tested without damage to functionality, are considered *in vivo* samples.

In an aspect of the present invention, the sample may be an *in vivo* human or animal sample, for example, but not limited to, a finger of a subject placed in an apparatus capable of changing temperature and analyzing the chemical composition of a sample by taking the spectrum of radiation affected by the sample. An example such an apparatus is disclosed in US 5,361,758 (which is incorporated herein by reference). In an alternate aspect of an embodiment of the present invention, the sample may be provided *in vitro*, for example, but not wishing to be limiting in any manner by placing blood or other body fluid taken from a subject into a cuvette or

other holder for analysis. Further, if the sample is an *in vitro* sample, then it may be subjected to processing prior to chemical composition analysis. Any processing step known in the art may be employed. Examples of processing steps may include, but are not limited to purification, centrifugation, separating contaminating or other chemical constituents prior to analysis, diluting, concentrating, reacting components in the sample with endogenous or exogenously added reagents or any combination thereof.

The method of the present invention may be employed to analyze any chemical component within a sample. Examples of chemical components may include, but are not limited to one or more proteins, sugars, lipids, and the like or any other component of organic or inorganic origin. Any physiological or nonphysiological chemical component in a biological or nonbiological sample may be analyzed by the method of the present invention.

The spectra which are used in the method of the present invention to analyze a chemical component in a sample or their physical state are obtained by exposing the sample to a suitable source of electromagnetic radiation, for example, but not limited to that is the infra-red, near infra-red, visible, and ultraviolet wavelength ranges. Preferably the source employs infrared or near infrared wavelengths.

As described above, the spectra of one or more than one compound of interest changes with a change in temperature. Furthermore, in samples comprising two or more compounds, the spectra of the sample changes with changes in temperature, in a manner that is distinct from the changes in the spectra of each compound separately. Therefore, these changes may be used to identify and characterize compounds of interest within a sample.

According to an aspect of the present invention, the method comprises generating a first spectrum of a sample at a first temperature, and obtaining one or more second spectra of the sample, at one or more second temperatures. Therefore, at least two or more spectra may be obtained from a sample. In the simplest method, only two spectra are obtained. Preferably, the first and second spectra are generated over a plurality of identical one or more wavelengths. Also contemplated by the method of the present invention, multiple spectra may be generated, and optionally

averaged at the first temperature, second temperature, or both. Further, the present invention contemplates generating spectra at more than two temperatures and using the information to determine the presence and concentration of a chemical component of interest within a sample and evaluating a thermal dynamic of the sample, thereby obtaining information on its physical state.

The first temperature and second temperature of the first and second spectra respectively, are different. Any temperature may be used in the present invention. For example, but not wishing to be limiting, the first temperature may be different from the second temperature, for example but not limited to, by about 0.1°C to about 500°C, or from about 1°C to about 100°C. In an aspect of the present invention, both the first and second temperatures are within the range of about 0°C to about 50°C, for example but not limited to about 20°C to about 40°C. However, temperatures outside this range are also contemplated and can be used for different samples, especially of non-organic origin. As would be evident to someone of skill in the art, preferably the temperatures are selected so that no damage occurs to the sample, for example, but not limited to denaturation of proteins, precipitation of chemical constituents, or destruction of the chemical component of interest in the sample, or causing discomfort to an individual or living creature.

The temperature of the sample may be adjusted by any means known in the art. For example, but not wishing to be limiting in any manner, the temperature of sample held within a cuvette or other holder may be adjusted by heating or cooling a chamber which holds the sample. In an alternate aspect of an embodiment, which is not meant to be limiting in any manner, an *in vivo* sample such as a finger or the like may be heated or cooled by the receptor device used to hold the sample, or by the beam source, for example, but not limited to an infrared beam including that employed for testing of sample in an infra-red portion of the spectrum. The beam may be the same or different from the electromagnetic radiation source used for the analysis of the sample, furthermore, microwave radiation can be used for sample heating.

In an aspect of the present invention, the first spectrum obtained for the sample at a first temperature is compared to the second spectrum at a second

temperature and the presence and amount of a chemical component of interest within the sample is determined. The comparison may involve computing the difference between the spectra or a point in the spectra. A calibration algorithm may be employed in the comparison, for example, but not limited to adjust for interfering or other components in a sample.

Alternatively, a certain number of spectra are registered at least at two different temperatures, the results averaged and standard deviation of mean value calculated. A ratio of the mean value to the standard deviation can be calculated (relative thermal spectral change ratio), and used for chemical analysis, or for investigation of structural changes in a sample that is related to temperature change. The ratio can be further processed in any known way, for example but not limited to smoothed normalized or normalized to a dynamic range of the instrument, as required.

As the spectra in samples comprising two or more compounds changes with temperature in a manner that is distinct from the changes in the spectra of each compound separately, and indicative of the compounds in the sample, then differences or the relative spectral thermal change between spectra obtained from the same sample at different or varying temperatures may be used to identify and characterize compounds of interest within an sample, or to observe structural changes of the sample. Such a method eliminates need for reference measurements of spectral content of radiation used for analysis and enables measurement, or increases the accuracy of the measurement, of a compound of interest. Further the method of the present invention does not require the use of a reference measurement, since by obtaining repeated spectra from the same sample, interfering factors are corrected for.

The above description is not intended to limit the claimed invention in any manner, Furthermore, the discussed combination of features might not be absolutely necessary for the inventive solution.

All references are herein incorporated by reference.

The present invention has been described with regard to preferred embodiments. However, it will be obvious to persons skilled in the art that a number

of variations and modifications can be made without departing from the scope of the invention as described herein.